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(54) Title: LIGANDS FOR Ga-68 PET HEART APPLICATIONS

(57) Abstract

Ligands and compositions for gallium-68 PET heart imaging are disclosed. The ligands form stable complexes with Ga-68. Variation of pendant substituents permits organ targeting with perfusion characteristics akin to a potassium mimic. Macrobicyclic, macrocyclic, and acyclic ligand designs are disclosed.

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LIGANDS FOR Ga-68 PET HEART APPLICATIONS

FIELD OF THE INVENTION

The present invention relates to radiodiagnostic imaging agents. More particularly, the present invention is directed to novel ligands and compositions for gallium-68 PET heart imaging.

BACKGROUND OF THE INVENTION

The development of radiopharmaceuticals for Positron Emission Tomography ("PET") for cardiac imaging based on flow, perfusion, and metabolism is a growing field of research. These radiopharmaceuticals include ^{11}C-glucose, ^{13}N-ammonia, ^{15}O-water, and ^{18}F-deoxyglucose. However, these short-lived isotopes (see Table 1) require the PET facility to be located very near a cyclotron dedicated to their production.

Table 1

Half-life of Positron Emitting Nuclides Used for PET-Heart Applications

Radionuclide	Half-life
^{11}C	20.40 min
^{13}N	9.96 min
^{15}O	2.04 min
^{18}F	109.80 min

Brown and Firestone, Table of Radioactive Isotopes, New York, Wiley, 1986.

The availability of a number of positron emitting isotopes which do not require production by means of an on site cyclotron is growing. These isotopes are produced by means of a generator system where a relatively long-lived parent decays to produce a daughter isotope which is then chemically separated from the production generator and made available for use in the production of potential new

radiopharmaceuticals. Generator-based radionuclides are attractive since they represent extremely transportable sources of radiopharmaceuticals. Thus, PET imaging could be made available to imaging centers across the country which are not located nearby an available cyclotron facility. Positron emitting radiopharmaceuticals based on generator produced isotopes would greatly enhance the versatility, and ultimately the utility, of any PET center.

Potentially useful generator produced isotopes are now widely available because of extensive development of their respective generator systems (see Table 2).

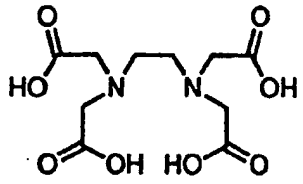
Table 2

Half-life of Positron Emitting Nuclide Generator Systems

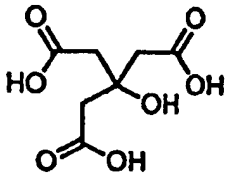
Parent/Daughter	Parent $T_{1/2}$	Daughter $T_{1/2}$
$^{68}Ge/^{68}Ga$	288 days	68.1 min
$^{82}Sr/^{82}Rb$	25 days	1.25 min
$^{52}Fe/^{52m}Mn$	8.28 hr	21.1 min
$^{122}Xe/^{122}I$	20.1 hr	3.6 min
$^{62}Zn/^{62}Cu$	9.2 hr	9.73 min
$^{44}Ti/^{44}Sc$	47 years	3.93 hr
$^{128}Ba/^{128}Cs$	2.43 days	3.6 min
$^{72}Se/^{72}As$	8.4 days	26 hr

The $^{82}Sr/^{82}Rb$ generator has been approved for routine clinical use. However, the short half-life of the daughter nuclide (1.25 minutes) limits the general utility of this isotope and the chemistry of $^{82}Rb^{+}$ prevents the application towards other organ specific imaging needs. The $^{68}Ge/^{68}Ga$ system is attractive since the parent nuclide is rather long-lived. This generator system is able to deliver clinically useful doses of ^{68}Ga for approximately eighteen months. After this length of time, the activity of ^{68}Ga eluted is too low for effective imaging.

Use of ^{68}Ga complexes as agents for organ specific PET imaging would require the development of suitable ligand systems to control biodistribution. Gallium-68 is chemically very similar to iron(III), and is readily taken up by plasma proteins such as transferrin. Thus, if a weakly coordinating ligand is used such as citrate or even EDTA, shown below, plasma protein labeling occurs, thereby restricting the radiolabel to the blood pool and resulting in slow clearance through the hepatobiliary system.



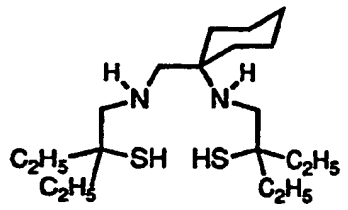
ethylenediaminetetraacetic acid
EDTA



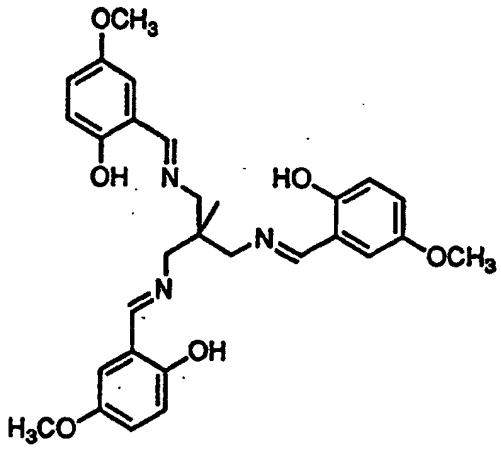
citric acid

A myocardial perfusion agent which incorporates ^{68}Ga, would have to be stable enough to prevent such exchange.

Recently, Kung et al., Journal of Nuclear Medicine, Vol. 31, p. 1635 (1990), demonstrated that a ^{68}Ga complex could be used as a myocardial imaging agent. The ligand used to deliver the radionuclide, BAT-TECH, below, to the heart did not present a stable coordination environment for the metal center. Liver uptake and very slow ^{68}Ga clearance of the blood pool were observed. Gallium-68 complexes of (5-MeOsal)$_3$TAME, below, have been evaluated as potential tracers of regional myocardial blood flow. (See, Green et al., Journal of Nuclear Medicine, Vol. 26, p. 170 (1985). But again, the design of the ligand gave undesirable clearance and biodistribution properties.



bis-aminoethylthiol-tetraethyl-cyclohexyl
BAT-TECH



tris(5-methoxysalicyaldiminomethyl)ethane
H_3[(5-MeOsal)$_3$TAME]

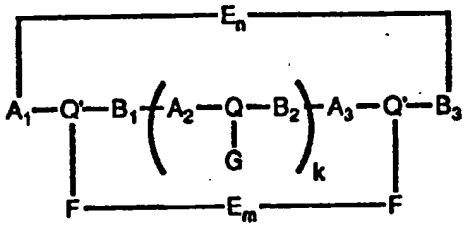
It will be appreciated that there is a need in the art for ligands which form stable complexes with Ga-68 and which contain suitable pendant substituents that permit organ targeting with perfusion characteristics akin to a potassium mimic.

BRIEF SUMMARY OF THE INVENTION

A principal goal of the ligands within the scope of the present invention is the ability to form compact, stable complexes with gallium-68 which behave as potassium mimics *in vivo*. It has been found that the basic diethylene triamine unit provides the basis for preparing suitable ligands within the scope of the present invention. Variations in the pendant ligating groups, amine design, and/or substituents placed throughout the ligand backbone, enable manipulation of key factors which influence myocardial uptake of labeled compounds, such as size, lipophilicity, charge distribution on the complex, and protein binding properties. Ethylene linkages, $-CH_2CH_2-$, are preferred between ligating groups because these provide the most stable metal chelating environment possible.

Larger spacings, e.g., propylene linkages, or constrained spacings, e.g. *ortho*-phenylene or 1,2-cyclohexyl, may be used, but may yield less-stable metal complexes.

The following is a generic ligand structure within the scope of the present invention capable of complexing Ga-68:



wherein A_x and B_x may be the same or different $-\overset{|}{C}R_1R_2$, where R_1 and R_2 may be oxygen, as in a carbonyl unit, or a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms; E_m and E_n are linking groups having the general formula: $-A-\overset{|}{Q}-B-$; n is from 0, 1 or 2, m is 0, 1 G or 2; k is 0, 1 or 2; G is a terminating unit having the general formula $-CR_1R_2R_3$, where R_1, R_2 and R_3 may a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms, R_3 is a metal binding group such as phenol, carboxylate, mercaptomethyl, or acetylacetonyl when E_m is not zero; F has the same general formula as A_x and B_x, above, but when E_m is zero, then F is a terminating unit having the general formula of G, where R_3 contains the metal

binding unit; when E_n is zero, then A_1 and B_3 are terminating units having the general formula of G, where R_3 is not a metal binding group; Q is an sp^3 nitrogen atom; Q' is an sp^3 or sp^2 nitrogen atom such that when Q' is an sp^2 nitrogen, A_1 and B_3 are 0, E_n and E_m are 0, k is 1, and F is a acetylacetonyl binding group.

As used herein, when the units E_n and E_m are "zero", then there is no bond or ring formed through A_1 and B_3 or the two F groups. Where k, n, or m is zero, then there is a bond connecting the respective adjacent groups A_x-B_y and F-F. The term C_0 is a hydrogen atom.

Ligand designs within the scope of the present invention can be divided into three general categories: (1) macrobicyclics, (2) macrocyclics, and (3) acyclics. These designs represent the simplest possible ligand format.

For example, according to the above generic formula, a typical macrobicyclic ligand (compound **1** of Figure 1) may be defined where k is 1, m is 0, n is 1, G is mercaptoethyl so that A_x, B_x, and F are $-CH_2-$ moieties. Another macrobicyclic ligand (compound **4** of Figure 1) may be defined where k is 2, m is 0, n is 0, G is mercaptoethyl so that A_x, B_x, and F are $-CH_2-$ moieties.

A typical macrocyclic ligand (compound **7** of Figure 2) may be defined according to the above generic formula where k is 1, E_m is 0, n is 0, F is mercaptoethyl, G is a terminating unit $-CR_1R_2R_3$ as "R" in Figure 2, and A_x and B_x and $-CH_2-$ moieties. Compounds **8** and **9** of Figure 2 are defined the same, except that F is a different metal binding group.

A typical acyclic ligand (compound **10** of Figure 3) may be defined according to the above generic formula where k is 1, E_m and E_n are 0, F is mercaptoethyl, A_1, G, and B_3 are terminating units $-CR_1R_2R_3$ as "R" in Figure 3. Compounds **11-13** of Figure 3 are defined the same, except that F is a different metal binding group.

Table 3 presents a summary of the currently preferred most stable ligands which are defined according to the above generic formula.

Table 3

Ligand Type	k	m	n	E_m	E_n
macrobicyclic	1	0	1	-	-
	2	0	0	-	-
macrocyclic	1	-	0	0	-
acyclic	1	-	-	0	0

General structures of possible macrobicyclic, macrocyclic, and acyclic ligands within the scope of the present invention are illustrated in Figures 1 through 3. Although the general structural formulas of Figures 1 through 3 do not illustrate the various substituents placed throughout the ligand backbone, it will be appreciated that such substituents may include hydrogen, alkyl, alcohol, ether, ester, carboxylic acid, thiol, sulfide, thiocarboxylic acid, amine, amide, hydroxy amine, phosphine, phosphite, phosphinate, or arene.

Radiolabeled species which are used as myocardial imaging agents for Single Photon Emission Tomography, SPECT, are typically small, lipophilic and monocationic. Such characteristics result in a complex which behaves as a potassium ion mimic. All of the proposed ligands within the scope of the present invention should yield very compact complexes of high stability. In all cases there are three or four metal binding amine residues and two anionic metal-binding moieties. Strategic placement of pendant groups, either as part of the aliphatic backbone or as substituents on non-pendant ligating amine atoms, will permit adjustment of complex lipophilicity. Since the aqueous chemistry of gallium is dominated exclusively by

oxidation state 3+, such designs will result in a complex which carries a monopositive charge.

From the foregoing, it will be appreciated that the present invention provides ligands which form stable complexes with Ga-68 and which contain suitable pendant substituents that permit organ targeting with perfusion characteristics akin to a potassium mimic.

BRIEF DESCRIPTION OF THE DRAWINGS

In order that the manner in which the above-recited and other advantages and features of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 illustrates typical macrobicyclic ligands for ^{68}Ga within the scope of the present invention.

Figure 2 illustrates typical macrocyclic ligands for ^{68}Ga within the scope of the present invention.

Figure 3 illustrates typical acyclic ligands for ^{68}Ga within the scope of the present invention.

Figure 4 illustrates different methods for synthesizing macrobicyclic ligands within the scope of the present invention.

Figure 5 illustrates different methods for synthesizing macrobicyclic ligands within the scope of the present invention.

Figure 6 illustrates different methods for synthesizing macrocyclic ligands within the scope of the present invention.

Figure 7 illustrates different methods for synthesizing acyclic ligands within the scope of the present invention.

Figure 8 illustrates a method for synthesizing an acyclic ligand within the scope of the present invention.

Figure 9 illustrates structural formulas for abbreviations used in Figures 4-8.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Ligands within the scope of the present invention are defined generically above. Various ligand designs, including macrobicyclic, macrocyclic, and acyclic ligands are possible according to the above generic definition. These designs represent the simplest possible ligand format. Variations in the pendant ligating groups, amine design, and/or substituents placed throughout the ligand backbone, enable manipulation of key factors which influence myocardial uptake of labeled compounds, such as size, lipophilicity, charge distribution on the complex, and protein binding properties. Ethylene linkages, $-CH_2CH_2-$, are preferred between ligating groups because these provide the most stable metal chelating environment possible. Larger spacings, e.g., propylene linkages, or constrained spacings, e.g. *ortho*-phenylene or 1,2-cyclohexyl, may be used, but may yield a less-stable metallocycle.

Examples of possible macrobicyclic, macrocyclic, and acyclic ligands within the scope of the present invention are illustrated in Figures 1 through 3. The macrobicyclic designs, compounds **1** through **6** of Figure 1, should produce the most stable complexes, since the metal center is effectively buried in the ligand "basket". A number of simple azabicyclics have been reported in the literature (Bencini et al., J. Chem. Soc. Chem. Comm., p. 174, 1990; Bencini et al., Inorg. Chem., Vol. 29, p. 3282, 1990;

Bencini et al., Inorg. Chem., Vol. 28, p. 4279, 1989; and Fortier et al., J. Amer. Chem. Soc., Vol. 112, p. 2640, 1990), but none with coordinating pendant groups. The amine precursor to ligands **1** through **3**, has been reported by Weisman et al., Tet. Lett., Vol. 21, p. 335, 1980. Synthesis of this moiety was accomplished by reaction of 1,4,7,10-tetrazacyclodecane with glyoxal. Recently, they have reported that reaction of the tetracyclic intermediate with methyl iodide, followed by reduction with sodium borohydride gave the dimethyl analogue of ligand 1, (Weisman et al., Presented at the 199th National Meeting of the American Chemical Society, Organic Chemistry Abstract #211, 1990). The bicyclic amine precursor to the simplest derivative for ligands 4 through 6 has been prepared, by Ramasubbu et al., J. Chem. Soc. Chem. Comm., p. 277, 1982, and its complexometric chemistry studied by Hancock et al., Inorg. Chem., Vol. 29, p. 264, 1990. A similar approach may be used to incorporate pendant ligating groups to produce ligands of the type **1** through **6**.

Examples of syntheses of macrobicyclic ligands within the scope of the present invention are illustrated in Figures 4 and 5.

The macrocyclic ligand designs, compounds **7** through **9** of Figure 2, though less encapsulating should still result in very strong complexes. Tris-substituted triazamacrocycles have been used to prepare neutral gallium complexes. Moore et al., Inorg. Chem., Vol. 29, p. 672, 1990. All such complexes were found to be very stable versus hydrolysis. Radiolabeled species were not prone to strong binding by blood proteins, i.e. loss of ^{68}Ga to transferrin. Moore et al., J. Nuc. Med., Vol. 30, p. 922, 1989 and Moore et al., J. Labelled Compd. Radiopharm., Vol 26, p. 362, 1989. Replacement of a pendant ligating groups by various R groups listed below will not reduce significantly the complex stability, but may add significantly to the

lipophilicity of the complex by disrupting outer sphere hydrogen bonding between the complex and endogenous water molecules. Proposed syntheses for macrocyclic ligands within the scope of the present invention are shown in Figure 6.

The acyclic ligand designs, compounds **10** through **13** of Figure 3, grant the greatest degree of freedom to the ligand and may present avenues by which decomplexation can occur, but these penta-coordinate designs should still provide a ligating environment strong enough to resist hydrolysis or trans-metallation with iron-binding blood proteins such as transferrin. Again, incorporation of various lipophilic R groups substituted at nitrogen or in the ethylene linking units of the ligand backbone may significantly impact the biodistribution and clearance behavior of the labeled complex. Proposed synthetic routes for acyclic ligands within the scope of the present invention are illustrated in Figures 7 and 8.

The ligands within the scope of the present invention may be complexed with Ga-68 and formulated in biocompatible solubilizing media for enteral or parenteral administration. The Ga-68 PET formulations may contain conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated.

For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of a Ga-68 ligand complex according to this invention. Various techniques for preparing suitable pharmaceutical solutions and suspensions are known in the art. Such solutions also may contain pharmaceutically acceptable buffers, stabilizers, antioxidants, and electrolytes, such as sodium chloride. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration.

Formulations for enteral administration may vary widely, as is well-known in the art. In general, such formulations include a diagnostically effective amount of a Ga-68 ligand complex in an aqueous solution or suspension. Such enteral compositions may optionally include buffers, surfactants, adjuvants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions within the scope of the present invention are administered in doses effective to achieve the desired PET image. Such doses may vary widely, depending upon the activity level of the Ga-68 generator, the organs or tissues which are the subject of the imaging procedure, the PET equipment being used, etc. Typical doses of the diagnostic compositions are in the range from about 0.1 to about 10 µmol/kg body weight, and preferably about 1 µmol/kg body weight.

The diagnostic compositions of this invention are used in a conventional manner in PET imaging procedures. Compositions may be administered in a sufficient amount to provide adequate visualization, to a warm-blooded animal either systemically or locally to an organ or tissues to be imaged, and the animal then subjected to the PET procedure.

The following examples are offered to further illustrate the present invention. These examples are intended to be purely exemplary and should not be viewed as a limitation on any claimed embodiment.

Example 1

Synthesis of 1,4-bis(p-toluenesulfonyl)-1,4-diazabutane, 1

In a 2L round bottom flask heated by a mantle and stirred mechanically, is placed ethylenediamine, 18g (0.30 mole), sodium carbonate, 64g (0.60 mole), and 750mL

distilled water. The resulting clear solution is heated to 75°C. To the reaction mixture is added *p*-toluenesulfonyl chloride, 125g (0.66 mole), in 5g batches over the course of a 45 minute time period. Once the full amount of acid chloride is added, the mixture is stirred at 75°C overnight. The mixture is cooled to room temperature, and the white precipitate is collected by filtration. The product is washed three times with distilled water, 250mL. The resulting white granular product is dried in a vacuum oven at 1mm and 45°C overnight. Yield 100.1g (98% based on ethylenediamine). Identity and purity determination is made by ^{1}H and ^{13}NMR, thin layer chromatography (5% methanol in chloroform as eluent on normal phase silica plates), and elemental analysis. Material produced in this fashion is satisfactory for use without further purification. If needed, the product is recrystallized from boiling acetonitrile.

Example 2

Synthesis of 1,5-bis(methanesulfonato)-3-methanesulfonyl-3-azapentane, 2

In a 2L round bottom flask fitted with a water cooled condenser, immersion thermometer, 250mL addition funnel, and magnetically stirred, is placed diethanolamine, 30g (0.29 mole), and 300mL pyridine. During the course of the reaction the reaction mixture is kept cool by means of a water-ice bath. To the cooled reaction mixture is added methanesulfonyl chloride, 73.4mL (108.6g, 0.95mole) in a dropwise fashion. The acid chloride is added at a rate so as to keep the reaction mixture temperature at or below 10°C. When the addition is complete, the mixture is warmed to room temperature and stirred overnight. The reaction mixture is cooled to 0° and 1L of 17% HCl, also at 0°C, is added. The brown precipitate which forms is collected and washed with distilled water until the filtrate is pH

neutral. The solid is dissolved in a minimum of warm, 35°C, 50:50 mixture of methanol:acetone. A colorless crystalline solid is deposited in the flask upon cooling. Yield 22g (25% based on diethanolamine). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (2% methanol in chloroform as eluent on normal phase silica plates), and elemental analysis.

Example 3

Synthesis of 1,4-bis(*p*-toluenesulfonyl)-7-methanesulfonyl-1,4,7-triazacyclononane, **3**

In a 500 mL round bottom flask fitted with a nitrogen flushed, water cooled condenser, 125mL addition funnel, heated by a mantle and stirred magnetically, is placed 1,4-bis(*p*-toluenesulfonyl)-1,4-diazabutane, **1**, 10g (0.030 mole), potassium carbonate, 8.5g (0.062 mole), and 100mL dry dimethylformamide. The slurry is stirred and heated to 35°C. To this mixture is added, via addition funnel, 1,5-bis(methanesulfonato)-3-methanesulfonyl-3-azapentane, **2**, 9.5g (0.031 mole) in 100mL dry DMF, in a dropwise fashion over a 3 hour time period. When the addition is complete, allow the mixture to stir, with heat, for 4 days. After removing the reaction solvent by rotary evaporation add 250g ice and shake vigorously. Collect the crude product by filtration and wash to neutral pH with distilled water. Place the crude product in a 500mL round bottom flask fitted with a water cooled reflux condenser, heated by mantle and magnetically stirred and add 200mL absolute ethanol. Reflux the slurry overnight and collect the white precipitate by filtration. Wash the white granular product with fresh absolute ethanol and air dry. Yield 12g (76% based on 1,4-bis(*p*-toluenesulfonyl)-1,4-diazabutane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (4% methanol in chloroform as

eluent on normal phase silica plates), and elemental analysis.

Example 4

Synthesis of methanesulfonyl-1,4,7-triazacyclononane, 4

In a 1L round bottom flask fitted with a water cooled, nitrogen flushed condenser, heated by mantle and stirred magnetically is placed 1,4-bis(*p*-toluenesulfonyl)-7-methanesulfonyl-1,4,7-triazacyclononane, 3, 38.8g (0.0752 mole), 300mL glacial acetic acid, and 150mL 48% hydrobromic acid. The mixture is heated to reflux for 72 hours. The wine red mixture is cooled to room temperature, and 90% of the reaction solvent is removed by means of rotary evaporation. 50mL 48% HBr is added, and the mixture is evaporated to a small volume again. This "washing" procedure is repeated twice more to remove all acetic acid. 100mL absolute ethanol and 200mL diethyl ether are added, and the mixture stirred. The pale tan precipitate is collected by filtration and washed with 3 x 100mL 50:50 absolute ethanol:diethyl ether. The product is air dried. The collected solid is placed in a 500mL round bottom flask fitted with a water cooled, nitrogen flushed condenser, heated by mantle and stirred magnetically. Sodium hydroxide pellets, 18g (0.45 mole) and 250mL toluene are added to the mixture. The mixture is refluxed overnight. The particulate solid present is removed by vacuum filtration and washed with 3 x 25mL fresh hot toluene. The combined filtrates are concentrated to approximately 60mL, by boiling, and allowed to cool slowly to room temperature. The colorless crystals are collected by vacuum filtration and washed with 2 x 10mL, 0°C, fresh toluene. The collected product is air dried. Yield 11.1g (71% based on 1,4-bis(*p*-toluenesulfonyl)-7-methanesulfonyl-1,4,7-triazacyclononane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (20%

ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 5

Synthesis of 4,10-bis(methanesulfonyl)-1,4,7,10-tetraazabicyclo-[5.5.2]-tetradecane), 5

In a 1L round bottom flask fitted with a water cooled, nitrogen flushed condenser, a 500mL addition funnel, heated by mantle and stirred magnetically is placed methanesulfonyl-1,4,7-triazacyclononane, 4; 5g (0.024 mole) potassium carbonate, 6.6g (0.048 mole) and 200mL dry N,N-dimethylformamide. The mixture is heated to 80°C and by means of the addition funnel, 1,5-bis(methanesulfonato)-3-methanesulfonyl-3-azapentane, 2, 9.0g (0.0265 mole), in 300mL dry N,N-dimethylformamide, is added in a dropwise fashion over a 6 hour time period. The mixture is stirred an additional three days upon completion of the addition. The volume of the mixture is reduced to a thick paste by means of a rotary evaporator. 500g ice are added to the residue and shaken thoroughly. The tan precipitate is collected by filtration and washed to neutral pH with fresh distilled water. The solid is transferred to a 500mL round bottom flask fitted with a water cooled condenser, heated by mantle and magnetically stirred. 250mL absolute ethanol are added, and the mixture is refluxed overnight. The precipitate is collected by filtration. The white granular solid is washed with fresh hot absolute ethanol and allowed to air dry. The solid is dissolved in a minimum of boiling 80:20 acetonitrile:absolute ethanol, filtered and allowed to cool slowly to room temperature. The colorless crystal is collected by filtration, washed with cold, 0°C, 50:50 acetonitrile:absolute ethanol, and allowed to air dry. Yield 5.7g (67% based on methanesulfonyl-1,4,7-triazacyclononane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer

chromatography (5% ethanol in ethyl acetate as eluent on normal phase silica plates), and elemental analysis.

Example 6

Synthesis of 1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane, 6

In a 250mL round bottom flask fitted with a water cooled, nitrogen flushed condenser, 125mL pressure equalized addition funnel, heated by mantle and stirred magnetically is placed 4,10-bis(methanesulfonyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane), 5g (0.014 mole) and 75mL dry tetrahydrofuran. 105mL of 3.4M solution of sodium bis(2-methoxyethoxy)aluminum hydride (RED-Al®) in toluene are slowly added by means of the addition funnel. When the addition is complete, the reaction is warmed to gentle reflux overnight. The volatiles are then removed from the reaction mixture by vacuum distillation, and the residue is cooled to ca. 10°C by means of a water-ice bath. 100mL water are slowly added, being careful not to allow the mixture to overheat or froth excessively. This mixture is acidified with concentrated hydrochloric acid to pH = 1. The mixture is then filtered to remove any particulate matter formed and transferred to a 1L separatory funnel. The aqueous solution is extracted with 3 x 250mL toluene. After transferring the aqueous layer to a 500mL Erlenmeyer flask, sodium hydroxide pellets are added to make pH = 14. The mixture is cooled, transferred to a separatory funnel, and extracted with 3 x 200mL toluene. The combined toluene extracts are dried with sodium sulfate, and the solution filtered to remove particular matter. After the solution is concentrated to 25mL and cooled to 0°C, the pale yellow crystals are collected and dried under vacuum. Yield 1.70g (61% based on 4,10-dimethanesulfonyl(1,4,7,10-tetraazabicyclo[5.5.2]tetradecane. Identify and purity determination is made by ^{1}H and ^{13}C NMR, thin layer

chromatography (15% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 7

Synthesis of 4,10-bis(2-mercaptoethyl)-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane·trihydrochloride, 7

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 124mL pressure equalized addition funnel, heated by mantle and stirred magnetically, containing 1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane, 6, 0.5g (0.0025) mole) in 25mL toluene is slowly added ethylene sulfide, 330µL (0.33g, 0.0055mole) in 25mL toluene. The reaction mixture is heated to 45°C during the addition. After the addition is complete, the mixture is stirred at 45°C for an additional 2 hours. The mixture is transferred to a clean 250mL round bottom flask, and all of the volatiles are removed by rotary evaporation. 100mL of a 50:50 mixture absolute ethanol:diethyl ether are added to the mixture, and the mixture is swirled. 15mL concentrated hydrochloric acid are slowly added, and the mixture is cooled to 0°C. The white flowery crystals are collected by filtration and washed with 2 x 50mL warm diethyl ether. Yield 0.95g (89% based on starting 1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane). Identity and purity determination is made by ^{1}H and ^{13}C NMR; mass spectroscopy, and elemental analysis.

Example 8

Synthesis of 4,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane, 8

To a 100mL round bottom flask, stirred magnetically and heated with a mantle to 40°C, containing 1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane, 6, 0.5g (0.0025 mole) in water is added bromoacetic acid, 0.7g (0.0055 mole),

dropwise. The pH of the reaction mixture is monitored and maintained at pH=13 with 5N sodium hydroxide solution. Once addition is complete, the mixture is allowed to stir an additional 3 hours, with the pH of the reaction mixture adjusted with 5N sodium hydroxide, as needed to maintain pH=13. The mixture is stirred an additional 3 hours after the pH has stabilized. The pH of the reaction mixture is then adjusted to pH=7.5 with 10% hydrochloric acid, and the mixture is evaporated by rotary evaporation. The hydrochloric acid is extracted, and the mixture is again evaporated by rotary evaporation. The residue is extracted with 3 x 250mL methanol. The combined methanol extracts are filtered and concentrated to 15mL volume by rotary evaporation. To this solution is added 25mL 1,2-dimethoxyethane and stirred overnight. The white product is collected by filtration. The yield is 0.49g (62% based on 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (10% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 9

Synthesis of 4,10-bis(2-hydroxybenzyl)-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane, 9

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel, heated by mantle, stirred magnetically, and containing 1,4,7,10-tetraazabicyclo-[5.5.2]tetradecane, 6, 0.5g (0.0025 mole) in 25mL methanol is added formaldehyde, as a 37% w/w solution, 2.41mL (0.45g, 0.0055 mole), and the mixture is allowed to stir at 40°C for three hours. Phenol, 0.52g (0.0055 mole), in 30mL methanol, is added dropwise to the yellow reaction mixture via the additional funnel. The mixture is stirred at 45°C

overnight. The reaction is transferred to a single neck 100mL round bottom flask, and all volatiles are removed from the reaction mixture by rotary evaporation. The residue is extracted with 3 x 50mL diethyl ether, and the combined extracts are dried with a small amount of magnesium sulfate. The mixture is filtered to remove the drying agent and concentrated to 10mL by boiling the solution. The mixture is slowly cooled to room temperature to affect crystallization of the product. Yield 0.30g (29% based on starting 1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (ethyl acetate as eluent on normal phase silica plates), and elemental analysis.

Example 10

Synthesis of 7,10-bis(mercaptoethyl)-1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane, **11**

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel, heated by mantle and stirred magnetically, containing 1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane, **10**, (prepared according to the procedure described by A. Ramasubbu et al., J. Chem. Soc. Chem. Comm., p. 277, 1982) 0.5g (0.0025 mole) in 15mL toluene is slowly added ethylene sulfide, 330µL (0.33g, 0.0055 mole) in 25mL toluene. The reaction mixture is heated to 45°C during the addition. When the addition is complete, the mixture is stirred at 45°C for an additional 2 hours. The mixture is then transferred to a clean 250 mL round bottom flask, and the volatiles are removed by rotary evaporation. 100mL of a 50:50 mixture absolute ethanol:diethyl ether are added and the mixture is swirled. 15mL of concentrated hydrochloric acid are slowly added, and the mixture is cooled to 0°C. The white flowery crystals are collected by

filtration and washed with 2 x 50mL warm diethyl ether. Yield 0.94g (87% based on starting 1,4,7,10-tetraazabicyclo[8.2.2]tetradecane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis.

Example 11

Synthesis of 7,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane, 12

To a 100mL round bottom flask, stirred magnetically and heated with a mantle to 40°C, containing 1,4,7,10-tetraazabicyclo[8.2.2]tetradecane, 10, (prepared according to the procedure described by A. Ramasubbu et al., J. Chem. Soc. Chem. Comm., p. 277, 1982) 0.5g (0.0025 mole) in water is added bromoacetic acid, 0.76g (0.0055 mole), dropwise. The pH of the reaction mixture is monitored and maintained at pH=13 with 5N sodium hydroxide solution. When the addition is complete, the mixture stirred an additional 3 hours, with the pH of the reaction mixture adjusted with 5N sodium hydroxide, as needed to maintain pH=13. The mixture is stirred an additional 3 hours, once pH has stabilized. The pH of the reaction mixture is then adjusted to pH=7.5 with 10% hydrochloric acid. The mixture is evaporated by rotary evaporation, and the residue is extract with 3 x 250mL methanol. The combined methanol extracts are filtered and then concentrated to 15mL volume by rotary evaporation. 25mL 1,2-dimethoxyethane is added to this solution and stirred overnight. The white product is collected by filtration. Yield 0.49g (62% based on 1,4,7,10-tetraazabicyclo[8.2.2]tetradecane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (10% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 12

Synthesis of 7,10-bis(2-hydroxybenzyl)-1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane, **13**

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel, heated by mantle stirred magnetically, and containing 1,4,7,10-Tetraazabicyclo-[8.2.2]tetradecane, **10**, (prepared according to the procedure described by A. Ramasubbu et al., J. Chem. Soc. Chem. Comm., p. 277, 1982) 0.5g (0.0025 mole) in 25mL methanol is added formaldehyde, as a 37% w/w solution, 0.4mL (0.45g, 0.0055 mole). The mixture is stirred at 40°C for three hours. Phenol, 0.52g (0.0055 mole), in 30mL methanol, is added dropwise via the addition funnel to the yellow reaction mixture. The mixture is stirred at 45°C overnight. The reaction mixture is transferred to a single neck 100mL round bottom flask, and all volatiles are removed from the reaction mixture by rotary evaporation. The residue is extracted with 3 x 50mL diethyl ether, and the combined extracts are dried with a small amount of magnesium sulfate. The mixture is filtered to remove the drying agent, and the filtrate is concentrated to 10mL by boiling the solution. The mixture is then cooled slowly to room temperature to affect crystallization of the product. Yield 0.30g (29% based on starting 1,4,7,10-tetraazabicyclo[8.2.2]-tetradecane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (ethyl acetate as eluent on normal phase silica plates), and elemental analysis.

Example 13

Synthesis of 1,5-bis(p-toluenesulfonato)-3-methyl-3-azapentane, **14**.

In a three-neck 2L round bottom flask fitted with a water cooled condenser, immersion thermometer, two 250mL

addition funnels, and magnetically stirred, is placed N-methyl-diethanolamine, 28.7mL (29.8g, 0.25 mole), and 100mL dichloromethane and 100mL distilled water. The pH of the aqueous layer is adjusted to pH=10. During the course of the reaction the reaction mixture is kept cool by means of a water-ice bath. To the cooled reaction mixture is added p-toluene-sulfonyl chloride, 104.9g (0.55 mole), in 150mL dichloromethane, in a dropwise fashion. Coincident to the addition of the acid chloride is added 25% w/w solution sodium hydroxide. The acid chloride and sodium hydroxide solutions are added at a rate so as to keep the pH of the aqueous reaction layer at pH=10. When the addition is complete, the mixture is warmed to room temperature and stirred for 2 hours. The organic layer is removed by siphon and treated with magnesium sulfate to dry. The solution is filtered to remove the magnesium sulfate. The dichloromethane is removed by rotary evaporation. The residue is dissolved in a minimum of warm, 35°C, 5:75:20 mixture of acetone:acetonitrile:hexane. A colorless crystalline solid is deposited in the flask upon cooling. Yield 66.3g (67% based on N-methyl-diethanolamine). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (methanol as eluent on normal phase silica plates), and elemental analysis.

Example 14

Synthesis of 1,4-bis(*p*-toluenesulfonyl)-7-methyl-1,4,7-triazacyclononane, 15.

To a three-neck 1L round bottom flask, fitted with a water cooled, nitrogen flushed condenser, 250mL pressure compensated addition funnel, heated by mantle, stirred magnetically and containing sodium hydride, 1.44g (0.060 mole), slurried in dry N,N-dimethylformamide, is added 1,4-bis(*p*-toluenesulfonyl)-1,4-diazabutane, 1, 10g (0.027 mole), in 100mL dry N,N-dimethylformamide, via the addition

funnel. During the course of the addition the reaction mixture is kept at 45°C. When the addition is complete, the mixture is stirred an additional two hours. To this warm mixture is added 1,5-bis(p-toluenesulfonato)-3-methyl-3-azapentane, 12.7g (0.030 mole), in 100mL dry N,N-dimethylformamide, dropwise, over a 6 hour period. When the addition is complete, the mixture is stirred an additional 72 hours. The reaction mixture is transferred to a clean 1L round bottom flask and concentrated to a thick paste by rotary evaporation. 250g ice are added, and the mixture is shaken vigorously. The crude product is collected by filtration and washed to neutral pH with distilled water. The crude solid is dried in vacuo (1 mmHg) and dissolved a minimum of boiling acetonitrile. The reaction mixture is filtered to remove solid impurities, and allowed to cool to room temperature slowly, to affect crystallization. Yield 8.3g, 68% (based on starting 1,4-bis(*p*-toluenesulfonyl)-1,4-diazabutane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (20% ethanol in ethyl acetate as eluent on normal phase silica plates), and elemental analysis.

Example 15

Synthesis of methyl-1,4,7-triazacyclononane, 16.

In a 500mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, heated by mantle and stirred magnetically, is placed 1,4-bis(*p*-toluenesulfonyl)-7-methyl-1,4,7-triazacyclonane, 15, 8g (0.018 mole), 150mL glacial acetic acid and 75mL 48% hydrobromic acid. The mixture is refluxed for 96 hours and concentrated by rotary evaporation. 100mL 48% hydrobromic acid is added to the residue, and the mixture is again concentrated by rotary evaporation. 10mL 48% hydrobromic acid and 100mL ethanol are added to the residue. The mixture is cooled to 5°C by means of an ice-water bath, and 300mL diethyl ether are

slowly added and mixed thoroughly. The off white precipitate is collected by filtration and washed with 2 x 50mL carbon tetrachloride and 4 x 100mL fresh diethyl ether. The solid is dried on the filter with suction and transferred to a 100mL round bottom flask. 75mL toluene are added. Sodium hydroxide pellets, 5g, are added, and the mixture is refluxed with a Dean-Stark trap for 12 hours. The hot mixture is filtered to remove the unreacted sodium hydroxide pellets and resulting sodium bromide. The filtrate is concentrated to 20mL volume and cooled to affect crystallization. Yield 2.3g, 91% (based on 1,4-bis(*p*-toluenesulfonyl)-7-methyl-1,4,7-triazacyclonane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (15% concentrated ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 16

Synthesis of 1,4-bis(2-mercaptoethyl)-7-methyl-1,4,7-triazacyclononane·dihydrochloride, **17**.

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel, heated by mantle and stirred magnetically, containing methyl-1,4,7-triazacyclononane, **16**, 0.5g (0.0035 mole) in 25mL toluene is slowly added ethylene sulfide, 460µL (0.46g, 0.0077 mole) in 25mL toluene. The reaction mixture is heated to 45°C during the addition. When the addition is complete, the mixture is stirred at 45°C for an additional 2 hours and transferred to a clean 250mL round bottom flask. All of the volatiles are removed by rotary evaporation. 100mL of a 50:50 mixture absolute ethanol:diethyl ether are added and swirled. 15mL concentrated hydrochloric acid are slowly added, and the mixture is cooled to 0°C. The white flowery crystals are collected by filtration and washed with 2 x

50mL warm diethyl ether. Yield 0.62g (48% based on starting methyl-1,4,7-triazacyclononane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis.

Example 17

Synthesis of 1,4-bis(carboxymethyl)-7-methyl-1,4,7-triazacyclononane, 18.

To a 100mL round bottom flask, stirred magnetically and heated with a mantle to 40°C, containing methyl-1,4,7-triazacyclononane, 16, 0.5g (0.0035 mole), in water is added bromoacetic acid, 1.1g (0.0077 mole), dropwise. The pH of the reaction mixture is monitored during the addition and maintained at pH=13 with 5N sodium hydroxide solution. When the addition is complete, the mixture is stirred an additional 3 hours, adjusting the pH of the reaction mixture with 5N sodium hydroxide, as needed to maintain pH=13. Once pH has stabilized, the mixture is stirred an additional 3 hours. The pH of the reaction mixture is then adjusted to pH-7.5 with 10% hydrochloric acid, and the mixture is evaporated by rotary evaporation. The residue is extracted with a 3 x 250mL methanol. The combined methanol extracts are filtered and concentrated to 15mL volume by rotary evaporation. To this solution, 25mL 1,2-dimethoxyethane are added, and the mixture is stirred overnight. The white product is collected by filtration. Yield 0.55 g (61% based on methyl-1,4,7-triazacyclononane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (10% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Page not transmitted by the receiving Office to the International Bureau at the time of publication

The reactor is then fitted with a Dean-Stark trap and refluxed overnight. Azeotropically distilled water, 7mL, is collected in the trap. The volatiles are removed by rotary evaporation, and the residue is dissolved in chloroform, 250mL. The mixture is concentrated to give an orange solid which is collected by vacuum filtration on a glass frit. The filtrate is concentrated to obtain more precipitate which is collected as before. The process is repeated a third time. The combined crops are slurried in acetonitrile, 300mL, and the resulting off-white solid is collected by filtration. The acetonitrile washing step is repeated two more times to give a nearly white powdery solid. Yield 47.7g (69.1% based on starting diethylenetriamine.) Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (5% methanol in chloroform as eluent on normal phase silica plates), and elemental analysis.

Example 20

Synthesis of 1,7-bis(phthalimido)-4-methyl-1,4,7-triazaheptane, 21.

In a 1L round bottom flask, fitted with a water cooled condenser, a 250mL additional funnel, an immersion thermometer, heated by mantle and stirred magnetically, is placed 1,7-bis(phthalimido)-1,4,7-triazaheptane, **20**, 30g (0.083 mole), potassium carbonate, 12.6g (0.091 mole), and acetonitrile, 300mL. The mixture is heated to 50°C. A solution containing iodomethane, 5.7mL (12.9g, 0.091 mole), in acetonitrile is added dropwise. The mixture is allowed to stir, with heating overnight. The volatiles are removed from the mixture by rotary evaporation, and the residue is extracted with chloroform, 250mL. The solution is filtered and concentrated to a volume 100mL. The mixture is cooled to 5-10°C to affect crystallization. The crystals are collected by vacuum filtration and washed with cold

acetone. Yield 18.2g (58% based on starting 1,7-bis(phthalimido)-1,4,7-triazaheptane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (2% methanol in chloroform as eluent on normal phase silica plates), and elemental analysis.

Example 21

Synthesis of 4-methyl-1,4,7-triazaheptane, 22.

In 500mL round bottom flask, fitted with a water cooled reflux condenser, heated by mantle and stirred magnetically, is placed 1,7-bis(phthalimido)-1,4,7-triazaheptane, **20**, 15g (0.040 mole), hydrazine, 35% w/w in water, 36mL (36.4g, 0.4 mole) and methanol, 225mL. The mixture is heated to reflux overnight. The mixture is then cooled to 35°C, and ammonium hydroxide is added. The mixture is stirred an additional 2 hours. The white precipitate is removed by vacuum filtration and washed thoroughly with 10% ammonium hydroxide in methanol. The filtrate is concentrated to a thick oil by rotary evaporation, and the residue is treated with toluene, 150mL, and magnesium sulfate, 15g. Allow the mixture to stand for 4 hours. The solids are removed by filtration, and the filtrate is transferred to 250mL round bottom flask for vacuum distillation. Vacuum distill the mixture at 1 mmHg and save the fraction distilling at 42-45°C. Yield 2.6g, (55% based on starting 1,7-bis(phthalimido)-1,4,7-triazaheptane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (25% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 22

Synthesis of 6-methyl-3,6,9-triazaundecane, 23.

In a three neck 250mL round bottom flask, fitted with a water cooled reflux condenser, 2 x 250mL addition

funnels, cooled in a water-ice bath and stirred magnetically, is placed 4-methyl-1,4,7-triazaheptane, **22**, 2g (0.017 mole), sodium hydroxide, 2g (0.050 mole), water, 50mL, and dichloromethane, 50mL. To this mixture is added a solution of acetyl chloride, 3mL (3.3g, 0.042mole) in dichloromethane, 25mL. Also added, at a rate so as to maintain pH>12, is a 0.5M solution of sodium hydroxide. When the addition is complete, the ice-water bath is removed, and the mixture is stirred for 3 hours. The pH of the aqueous layer is maintained greater than 12, and the mixture is transferred to a 500mL separatory funnel. The organic layer is collected and washed with 3 x 100mL, 10% sodium hydroxide. The organic layer is collected and treated with 5g magnesium sulfate overnight. The solids are removed from the mixture by filtration, and the filtrate is evaporated to a thick oil. The residue is extracted with dry tetrahydrofuran and transferred to a nitrogen flushed round bottom flask, fitted with a nitrogen flushed pressure compensated addition funnel. Borane-tetrahydrofuran complex, 1M in tetrahydrofuran, 50mL, is added dropwise via the addition funnel. The reducing agent is added at a rate which will not heat the mixture. The mixture is stirred overnight. The addition funnel is removed, under nitrogen purge, and a nitrogen flushed, water cooled condenser is attached. The mixture is heated to reflux for two hours and then cooled to room temperature. The volatiles are removed by rotary evaporation. Hydrochloric acid, 200mL 10% solution, is carefully added with rapid stirring. The mixture is filtered to remove any solids formed, and the pH of the solution is adjusted to pH=13 with sodium hydroxide pellets. The mixture is cooled to room temperature, transferred to a separatory funnel, and extracted with 3 x 100mL diethyl ether. The combined ether fractions are treated with 20g magnesium sulfate and allowed to stand

overnight. The mixture is filtered to remove the solids present. The filtrate is transferred to a 500mL round bottom flask for vacuum distillation. Upon vacuum distillation at 1 mmHg, the fraction distilling at 48-52°C is saved. Yield 2.2g, 76% based on starting 4-methyl-1,4,7-triazaheptane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (25% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 23

Synthesis of 3,9-bis(2-mercaptoethyl)-6-methyl-3,6,9-triazaundecane·dihydrochloride, **24**.

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel, heated by mantle and stirred magnetically, containing 6-methyl-3,6,9-triazaundecane, **23**, 0.5g (0.0029 mole) in 25mL toluene is slowly added ethylene sulfide, 380µL (0.38g, 0.0064 mole) in 25mL toluene. The reaction mixture is heated to 45°C during the addition. When the addition is complete, the mixture is stirred at 45°C for an additional 2 hours and then transferred to a clean 250mL round bottom flask. All of the volatiles are removed by rotary evaporation. 100mL of a 50:50 mixture absolute ethanol:diethyl ether are added, and the mixture is swirled. 15mL concentrated hydrochloric acid are slowly added, and the mixture is cooled to 0°C. The white flowery crystals are collected by filtration and washed with 2 x 50mL warm diethyl ether. Yield 0.85g (80% based on starting 6-methyl-3,6,9-triazaundecane). Identity and purity determination is made by ^{1}H and ^{13}C NMR, mass spectroscopy, and elemental analysis.

Example 24

Synthesis of 3,9-bis(carboxymethyl)-6-methyl-3,6,9-triazaundecane, **25**.

To a 100mL round bottom flask, stirred magnetically and heated with a mantle to 40°C, containing 6-methyl-3,6,9-triazaundecane, **23**, 0.5g (0.0029 mole), in water is added bromoacetic acid, 0.88g (0.0064 mole), dropwise. The pH of the reaction mixture is monitored and maintained at pH=13 with 5N sodium hydroxide solution. When the addition is complete, the mixture is stirred an additional 3 hours, adjusting the pH of the reaction mixture with 5N sodium hydroxide, as needed to maintain pH=13. Once pH has stabilized, the mixture is stirred an additional 3 hours. The pH of the reaction mixture is adjusted to pH=7.5 with 10% hydrochloric acid, and the mixture is evaporated by rotary evaporation. The residue is extracted with 3 x 250mL methanol. The combined methanol extracts are filtered and concentrated to 15mL volume by rotary evaporation. 1,2-dimethoxyethane, 25mL, is added to this solution and stirred overnight. The white powdery product is collected by filtration. Yield 0.52g (62% based on 6-methyl-3,6,9-triazaundecane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (10% ammonium hydroxide in methanol as eluent on normal phase silica plates), and elemental analysis.

Example 25

Synthesis of 3,9-bis(2-hydroxybenzyl)-6-methyl-3,6,9-triazaundecane, **26**.

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized additional funnel, heated by mantle stirred magnetically and containing 6-methyl-3,6,9-triazaundecane, **23**, 0.5g (0.0029 mole), in 25mL methanol is added

formaldehyde, as a 37% w/w solution, 0.47mL (0.52g, 0.0064 mole), and the mixture allowed to stir at 40°C for three hours. Phenol, 0.60g (0.0064 mole), in 30mL methanol, is added dropwise to the yellow reaction mixture via the addition funnel. The mixture is stirred at 45°C overnight and transferred to a single neck 100mL round bottom flask. All volatiles are removed from the reaction mixture by rotary evaporation. The residue is extracted with 3 x 50mL diethyl ether. The combined extracts are dried with a small amount of magnesium sulfate. The mixture is filtered to remove the drying agent, and the filtrate is concentrated to 10mL by boiling the solution. The mixture is cooled slowly to room temperature to affect crystallization of the product. Yield 0.30g (27% based on starting methyl-1,4,7-triazacyclononane. Identity and purity determination is made by ^{1}H and ^{13}C NMR, thin layer chromatography (ethyl acetate as eluent on normal phase silica plates), and elemental analysis.

Example 26

Synthesis of 1,7,-bis(3-pentene-2-one-4-yl)-4-methyl-1,4,7-triazaheptane, **27**.

To a two-neck 100mL round bottom flask, fitted with a water cooled, nitrogen flushed, condenser, 125mL pressure equalized addition funnel heated by mantle and stirred magnetically, containing 4-methyl-1,4,7-triazaheptane, **22**, 0.5g (0.0043 mole) in 25mL diethyl ether is slowly added acetylacetone 0.96mL (0.94g, 0.094 mole) in 25mL diethyl ether. An immediate precipitate forms. The mixture is heated to reflux overnight. The reaction mixture is cooled to room temperature, and the yellow solid is collected by filtration. The solid powder is washed with 2 x 50mL fresh diethyl ether and dried with suction on the filter pad. The solid is dissolved in a minimum of boiling 50:50 acetone:ethyl acetate. Yield 1.1g (89% based on starting

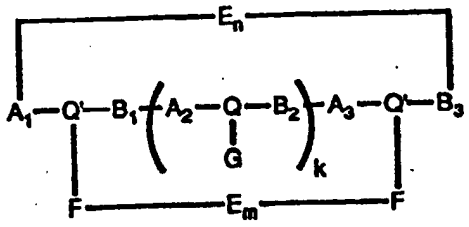
4-methyl-1,4,7-triazaheptane). Identity and purity determination is made by ^{1}H and ^{13}C NMR. thin layer chromatography (50% ethyl acetate in hexanes as eluent on normal phase silica plates), and elemental analysis.

From the foregoing, it will be appreciated that the present invention provides ligands which form stable complexes with Ga-68 and which contain suitable pendant substituents that permit organ targeting with perfusion characteristics akin to a potassium mimic.

The invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

What is claimed is:

1. A ligand capable of complexing Ga-68 having a generic formula:



wherein A_x and B_x may be the same or different $-CR_1R_2$, where R_1 and R_2 may be oxygen, as in a carbonyl unit, or a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms; E_m and E_n are linking groups having the general formula: -A-Q-B-; n is from 0, 1 or 2, m is 0, 1 or 2; k is 0, 1 or 2; G is a terminating unit having the general formula $-CR_1R_2R_3$, where R_1, R_2 and R_3 may a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms, R_3 is a metal binding group such as phenol, carboxylate, mercaptomethyl, or acetylacetonyl when E_m is not zero; F has the same general formula as A_x and B_x, above, but when E_m is zero, then F is a terminating unit having the general formula of G, where R_3 contains the metal binding unit; when E_n is zero, then A_1 and B_3 are terminating units having the general formula of G, where R_3 is not a metal binding group; Q is an sp^3 nitrogen atom; Q' is an sp^3

or sp^2 nitrogen atom such that when Q' is a [illegible] nitrogen, A_1 and B_3 are 0, E_n and E_m are 0, k is [illegible] and F is a acetylacetonyl binding group.

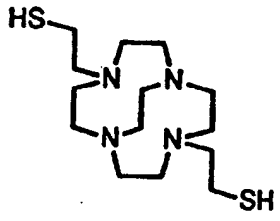
2. A ligand as defined in claim 1, [illegible] k is 1, m is 0, n is 1, and G is a metal binding gr[illegible].

3. A ligand as defined in claim 1, [illegible] k is 2, m is 0, n is 0, and G is a metal binding gr[illegible]

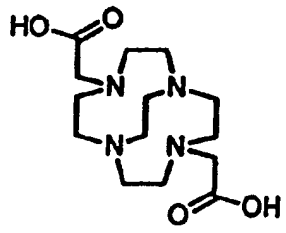
4. A ligand as defined in claim 1, [illegible] k is 1, E_m is 0, n is 0, and F is a metal binding g[illegible].

5. A ligand as defined in claim 1, [illegible] k is 1, E_m is 0, E_n is 0, F is a metal binding grou[illegible] A_1 and B_3 are terminating units.

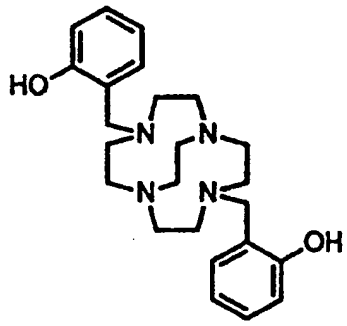
6. A ligand as defined in claim [illegible] having the following general structure:



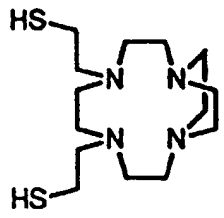
7. A ligand as defined in claim 2 having the following general structure:



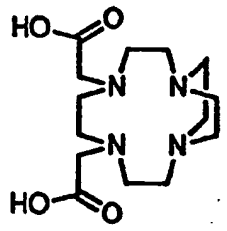
8. A ligand as defined in claim 2 having the following general structure:



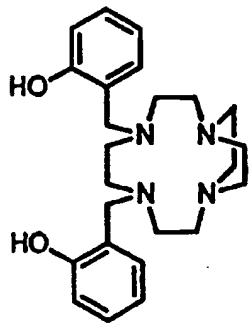
9. A ligand as defined in claim 3 having the following general structure:



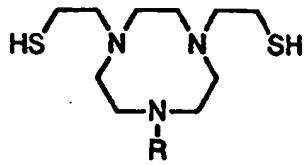
10. A ligand as defined in claim 3 having the following general structure:



11. A ligand as defined in claim 3 having the following general structure:

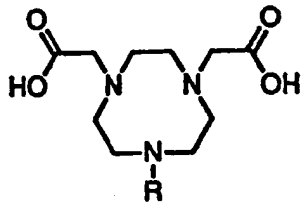


12. A ligand as defined in claim 4 having the following general structure:



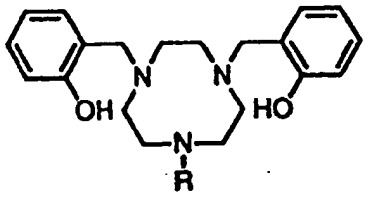
wherein R is a terminating unit having the general formula of G, above, where R_3 is not a metal binding group.

13. A ligand as defined in claim 4 having the following general structure:



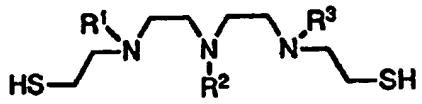
wherein R is a terminating unit having the general formula of G, above, where R_3 is not a metal binding group.

14. A ligand as defined in claim 4 having the following general structure:



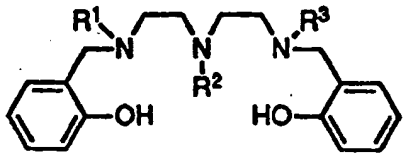
wherein R is a terminating unit having the general formula of G, above, where R_3 is not a metal binding group.

15. A ligand as defined in claim 5 having the following general structure:



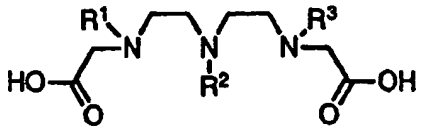
wherein R^1, R^2, and R^3 are terminating units having the general formula of G, above, where R_3 is not a metal binding group.

16. A ligand as defined in claim 5 having the following general structure:



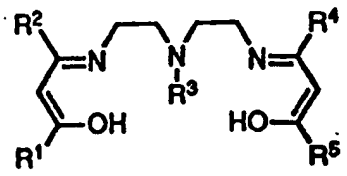
wherein R^1, R^2, and R^3 are terminating units having the general formula of G, above, where R_3 is not a metal binding group.

17. A ligand as defined in claim 5 having the following general structure:



wherein R^1, R^2, and R^3 are terminating units having the general formula of G, above, where R_3 is not a metal binding group.

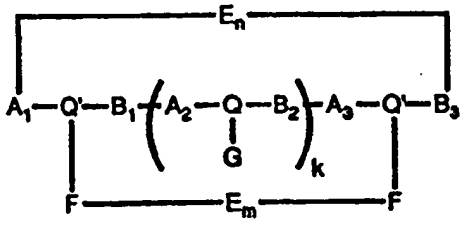
18. A ligand as defined in claim 5 wherein A_1 and B_3 are O and F is an acetylacetonyl binding group, having the following general structure:



wherein R^1, R^2, R^3, R^4, and R^5 are terminating units having the general formula of G, above, where R_3 is not a metal binding group.

19. A method for obtaining a PET image of heart tissues comprising the steps of:

(a) administering to a warm-blooded animal a diagnostically effective amount of a gallium-68 complex with a ligand having a generic formula:



wherein A_x and B_x may be the same or different $-\overset{|}{C}R_1R_2$, where R_1 and R_2 may be oxygen, as in a carbonyl unit, or a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms; E_n

and E_n are linking groups having the general formula: -A-Q(G)-B-; n is from 0, 1 or 2, m is 0, 1 or 2; k is 0, 1 or 2; or 2; k is 0, 1 or 2; G is a terminating unit having the general formula $-CR_1R_2R_3$, where R_1, R_2 and R_3 may a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms, R_3 is a metal binding group such as phenol, carboxylate, mercaptomethyl, or acetylacetonyl when E_m is not zero; F has the same general formula as A_x and B_x, above, but when E_m is zero, then F is a terminating unit having the general formula of G, where R_3 contains the metal binding unit; when E_n is zero, then A_1 and B_3 are terminating units having the general formula of G, where R_3 is not a metal binding group; Q is an sp^3 nitrogen atom; Q' is an sp^3 or sp^2 nitrogen atom such that when Q' is an sp^2 nitrogen, A_1 and B_3 are 0, E_n and E_m are 0, k is 1, and F is a acetylacetonyl binding group; and

(b) imaging the animal's heart tissues.

20. A method for obtaining a PET image of heart tissues as defined in claim 19, wherein the gallium-68 is complexed with a ligand formula in which k is 1, m is 0, n is 1, and G is a metal binding group.

21. A method for obtaining a PET image of heart tissues as defined in claim 19, wherein the gallium-68 is complexed with a ligand formula in which k is 2, m is 0, n is 0, and G is a metal binding group.

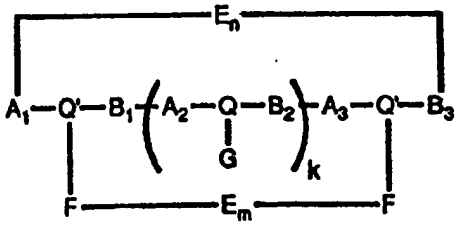
22. A method for obtaining a PET image of heart tissues as defined in claim 19, wherein the gallium-68 is complexed with a ligand formula in which k is 1, E_m is 0, n is 0, and F is a metal binding group.

23. A method for obtaining a PET image of heart tissues as defined in claim 19, wherein the gallium-68 is complexed with a ligand formula in which k is 1, E_m is 0, E_n is 0, F is a metal binding group, and A_1 and B_3 are terminating units.

24. A method for obtaining a PET image of heart tissues as defined in claim 19, wherein the diagnostically effective amount of the gallium-68 complex is administered in a dose range from about 0.1 to about 10 µmol/kg animal body weight.

25. A diagnostic composition suitable for enteral or parenteral administration to a warm-blooded animal comprising:

a diagnostically effective amount of a gallium-68 complex with a ligand having a generic formula:



wherein A_x and B_x may be the same or different $-\overset{|}{C}R_1R_2$, where R_1 and R_2 may be oxygen, as in a carbonyl unit, or a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid,

amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms; E_m and E_n are linking groups having the general formula: -A-Q(G)-B-; n is from 0, 1 or 2, m is 0, 1 or 2; k is 0, 1 or 2; or 2; k is 0, 1 or 2; G is a terminating unit having the general formula $-CR_1R_2R_3$, where R_1, R_2 and R_3 may a C_0-C_4 substituted alkyl chain having as substituents hydrogen, alkyl, hydroxyl, ether, ester, carboxylic acid, thiol, sulfide, thio-carboxylic acid, amine, amide, hydroxyamine, phosphine, phosphite, phosphinate, or arene wherein the carbon-containing substituent groups have from 1 to 4 carbon atoms, R_3 is a metal binding group such as phenol, carboxylate, mercaptomethyl, or acetylacetonyl when E_m is not zero; F has the same general formula as A_x and B_x, above, but when E_m is zero, then F is a terminating unit having the general formula of G, where R_3 contains the metal binding unit; when E_n is zero, then A_1 and B_3 are terminating units having the general formula of G, where R_3 is not a metal binding group; Q is an sp^3 nitrogen atom; Q' is an sp^3 or sp^2 nitrogen atom such that when Q' is an sp^2 nitrogen, A_1 and B_3 are 0, E_n and E_m are 0, k is 1, and F is a acetylacetonyl binding group; and

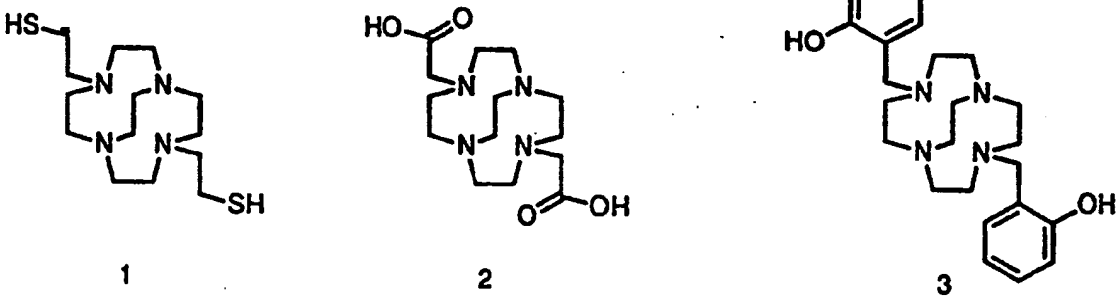
a pharmaceutically acceptable carrier.

26. A diagnostic composition as defined in claim 25, wherein the gallium-68 is complexed with a ligand formula in which k is 1, m is 0, n is 1, and G is a metal binding group.

27. A diagnostic composition as defined in claim 25, wherein the gallium-68 is complexed with a ligand formula in which k is 2, m is 0, n is 0, and G is a metal binding group.

28. A diagnostic composition as defined in claim 25, wherein the gallium-68 is complexed with a ligand formula in which k is 1, E_m is 0, n is 0, and F is a metal binding group.

29. A diagnostic composition as defined in claim 25, wherein the gallium-68 is complexed with a ligand formula in which k is 1, E_m is 0, E_n is 0, F is a metal binding group, and A_1 and B_3 are terminating units.



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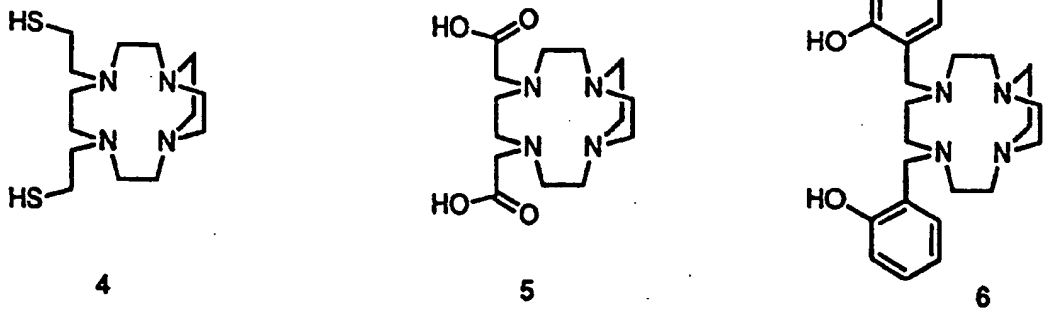
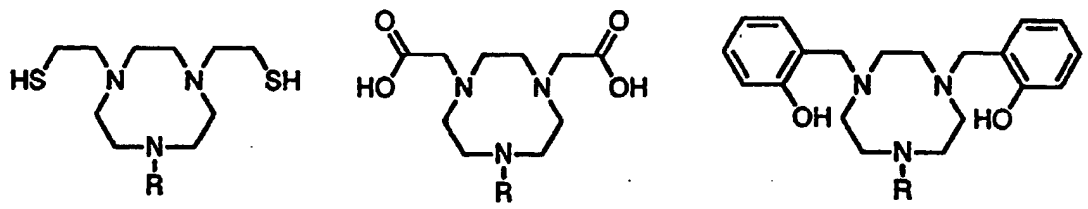


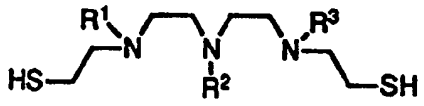
4 5 6

FIG. 1



7 8 9

FIG. 2



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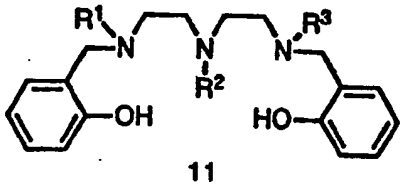

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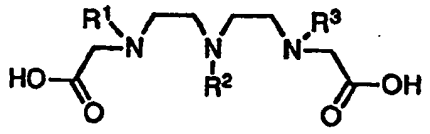

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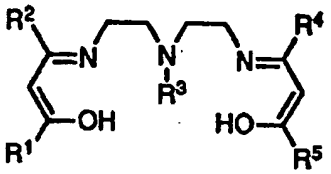


13

FIG. 3

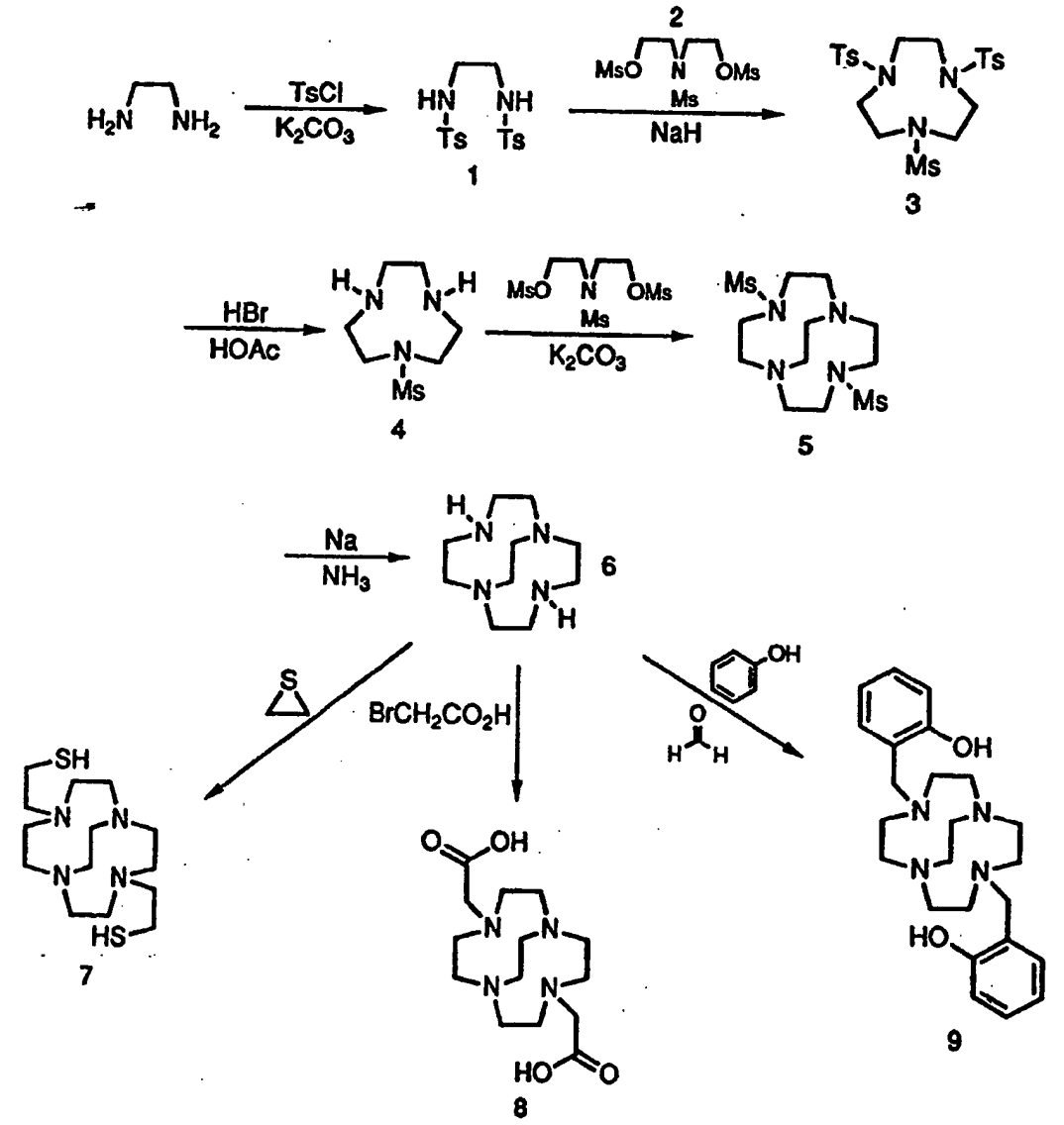


FIG. 4

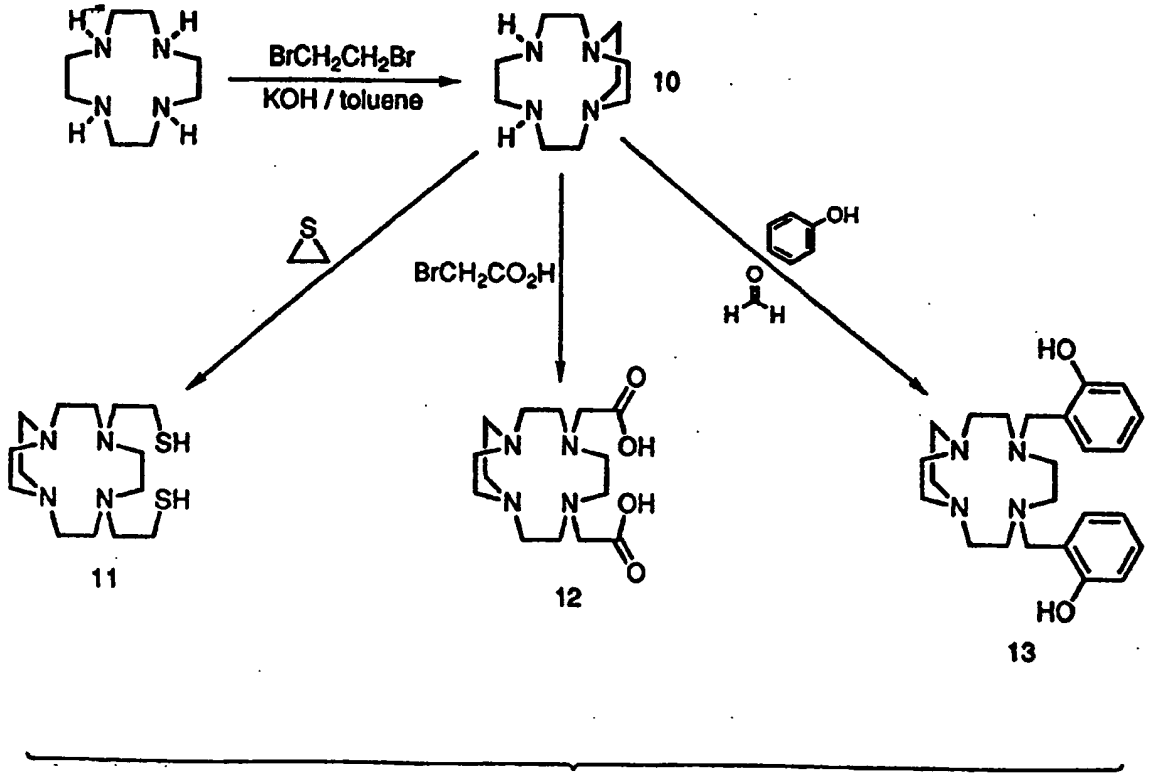
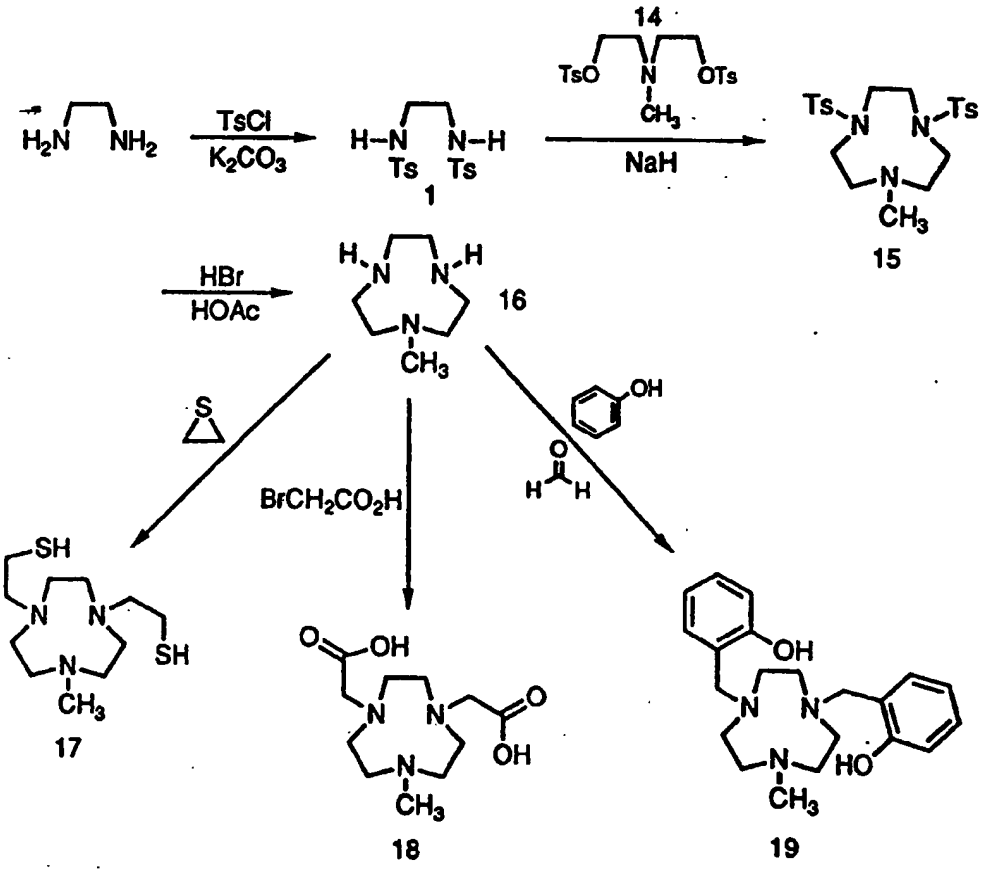


FIG. 5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TsCl
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NH2
H-N
N-H
Ts
Ts
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N
N
Ts
N
CH3
15
HBr
HOAc
H
N
N
H
16
N
CH3
S
OH
O
H
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BrCH2CO2H
SH
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N
SH
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N
HO
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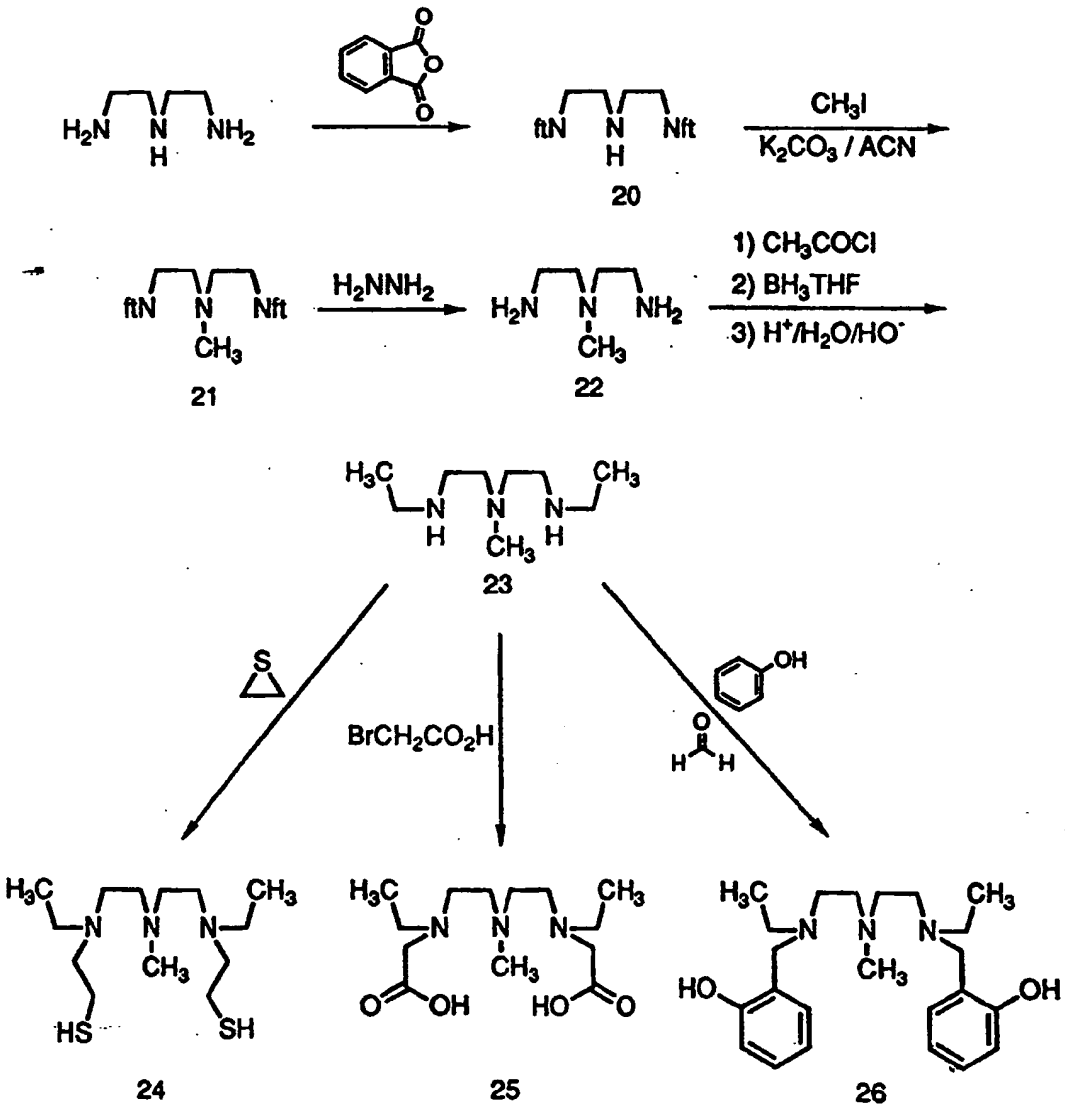
CH3
19

FIG. 6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ftN N Nft
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CH3I
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ftN N Nft
CH3
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CH3
22
1) CH3COCl
2) BH3THF
3) H+/H2O/HO-
H3C N N N CH3
H H CH3
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OH
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H H
HS SH
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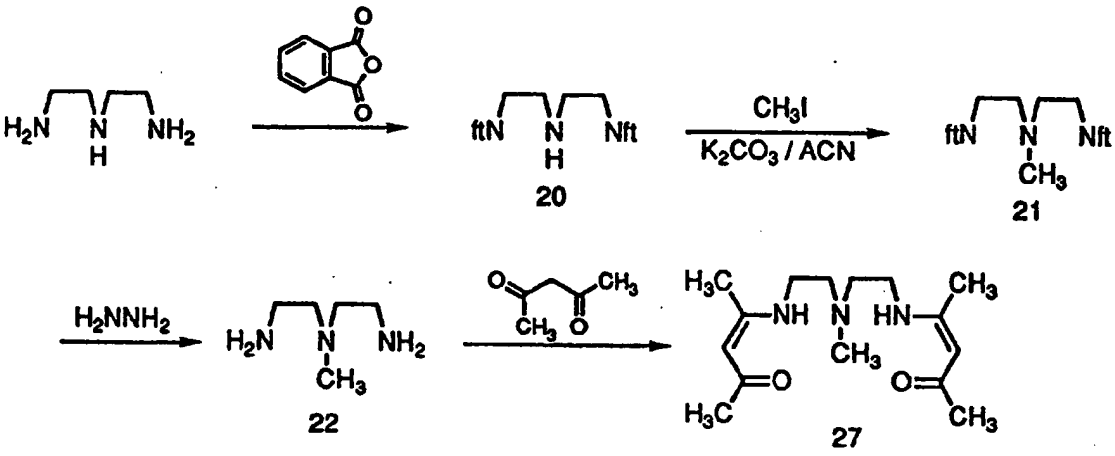
OH O HO O
25
HO OH
26

FIG. 7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CH3
CH3 O
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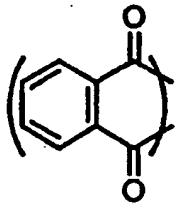
CH3
O O
H3C CH3
27

FIG. 8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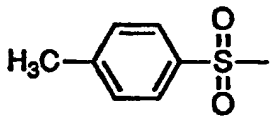
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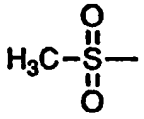
= Ms

= ACN

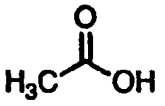
= HOAc

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07769

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07C 229/76; C07F 5/00, 9/38, 13/00,15/02; A61K 49/00

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS.ONLINE structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,957,939 (Gries et al) 18 September 1990, see entire document.	1-29
Y	EP, A, 287,465 (Schaeffer) 19 October 1988, see entire document.	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 NOVEMBER 1993	NOV 16 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CECILIA TSANG Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07769

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Inorg. Chem., Vol.29,issued 1990, Ulf Auerbach et al, " Synthesis and Coordination Chemistry of the Hexadentate Ligands 1,4,7-Tris(2-hydroxybenzyl)-1,4,6-triazacyclononane (H3L1) and 1,4,7-Tris(3-tert-butyl-2-hydroxybenzyl)-1,4,7-triazacyclononane (H3L2). Crystal Structures of [HL1CuII] and [L2FeIII]acacH.", pages 938-944.	1-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07769

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

540/465, 472,474; 424/1.1, 4, 9, 11, 85, 131, 140, 144, 147; 562/590; 564/348, 336; 514/6, 79, 108, 150, 184, 492, 497-499, 501-503, 505; 534/15, 16

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

540/465, 472, 474; 424/1.1, 4, 9, 11, 85, 131, 140, 144, 147; 562/590; 564/348, 336; 514/6, 79, 108, 184, 492, 497-499, 501-503, 505; 534/15, 16